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Ornithine Decarboxylase Exhibits Negative Thermal Modulation in the Sea Star *Asterias vulgaris:* Potential Regulatory Role During Temperature-Dependent Testicular Growth

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Abstract. The common northern sea star *Asterias vulgaris* is exposed to seasonal variation in temperature from −2 to 17°C. *A. vulgaris* exhibits an annual reproductive cycle, *i.e.*, the testes increase slowly in size during fall and winter, and reach maximal size in early spring. Slow testicular growth in the winter has been attributed to low field temperatures. Previous studies indicate that the specific activity of ornithine decarboxylase and the levels of the polyamines putrescine, spermidine, and spermine decrease in mid-winter and increase in the spring, coincident with changes in field temperatures. Kinetic studies show that ornithine decarboxylase assayed from individuals collected in March exhibits negative thermal modulation (K_m of ornithine is 0.22 m*M* and 0.65 m*M* at 15 and 0°C, respectively). Q_{10} values are highest at low substrate concentrations and at low temperatures. We hypothesize that during the cold winter months a decrease in the amount of ODC and an increase in the apparent K_m causes polyamine synthesis to decline, leading to decreased growth and development of the testis. We suggest that thermal modulation of ODC (and polyamine synthesis) is a mechanism by which seasonal temperature fluctuations influence seasonal spermatogenesis in *A. vulgaris*. We further suggest that growth of various tissues in many other ectothermal invertebrates may be similarly controlled.

Introduction

The biogenic polyamines spermidine and spermine and their diamine precursor putrescine are organic cations with multiple biological functions. Polyamines are known principally for their essential role in cell proliferation and for interactions with anionic molecules such as nucleic acids and membrane phospholipids in mammalian cell lines and tissues, both normal and neoplastic. However, the exact mechanisms by which polyamines influence cell proliferation are not fully understood.

Polyamine synthesis is controlled via the rate-limiting enzyme ornithine decarboxylase (ODC) (1). The activity of mammalian ODC varies in response to a wide variety of stimuli including growth factors, hormones, and drugs (2). The inducibility and short half life of ODC [<15 min, (1)] suggest that the activity of the enzyme (and thus polyamine synthesis) is highly regulated. The activity and kinetics of mammalian ODC have been characterized in numerous studies (reviewed by ref. 1). ODC has not been characterized for any invertebrate with the exception of a partial kinetic analysis of the enzyme in a snail (3).

The role of ODC and polyamines during cell proliferation in invertebrates should be similar to cell proliferation in mammals. However, relatively few studies have examined polyamine metabolism in the tissues of invertebrates. This is unfortunate because many invertebrates, particularly larvae and juveniles, show high seasonal and yearly growth rates. Polyamines are present in tissues of several invertebrates, although their distribution varies greatly among tissue types (4, 5, 6, 7, 8, 9, 10). In addition, polyamine levels increase significantly during periods of mitotic and meiotic cell proliferation in the testes of the sea star *Asterias vulgaris* (11). Cell proliferation in the testes, demonstrated by thymidine incorporation, also increases during exposure to extrinsically applied polyamines (12).

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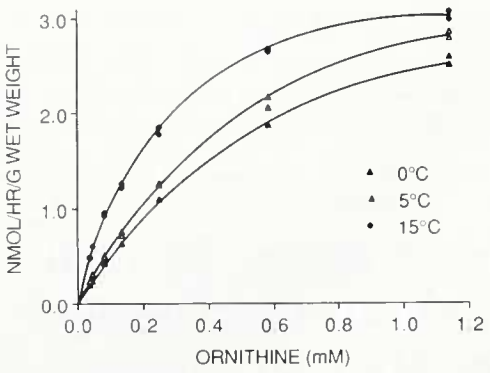

Figure 1. Activity of ornithine decarboxylase measured at various substrate (ornithine) concentrations and at assay temperatures of 0, 5, and 15°C. All assays were performed in duplicate.

Many invertebrates are exposed to daily or seasonal changes in temperature and demonstrate temperature-dependent growth characteristics. Although temperature is often cited as an important modulator of growth, the mechanisms by which temperature-dependent growth is regulated remain obscure. We hypothesize that temperature regulation of ornithine decarboxylase activity and polyamine synthesis may be one mechanism by which temperature regulates growth in ectothermal organisms. In this study we report the effect of assay temperature on the kinetic characteristics of ornithine decarboxylase extracted from the testes of *A. vulgaris*.

Materials and Methods

Adult specimens of *Asterias vulgaris* (8–10 cm arm length) were collected from a depth of 3 meters at the mouth of the Piscatequa River in Portsmouth, New Hampshire, in March 1987. Testes were removed from three individuals and pooled for ODC extraction and analysis.

Fresh testes were homogenized (20% w:v, 1:4) on ice with a glass Teflon homogenizing apparatus in a buffer containing 50 m*M* KH_2PO_4, pH 7.5, 0.2 m*M* EDTA, 5 m*M* dithiothreitol and 50 μM pyridoxal 5-phosphate. The crude extract was centrifuged for 30 min at 20,000 $\times g$ at 0°C. The supernatant was used for enzyme activity determinations.

The specific activity of ODC was determined by a procedure modified from Landy-Otsuka and Scheffler (13) and Smith (14). The specific activity of ODC was determined by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C] ornithine hydrochloride (CFA.423, Amersham, 58 mCi/mmol). The enzyme reaction was performed in a 16 mm (ID) borosilicate test tube capped with a double-seal rubber stopper (Kontes, K-882310) penetrated by a plastic centerwell (Kontes K-882320). The centerwell contained a 2 × 3 cm square of Whatman #1 filter paper saturated with 100 μl NCS tissue solubilizer (Amersham). First, 150 μl of the above supernatant was added to each tube, and the reaction was initiated by adding 30 μl of 0.5 μCi DL-[1-^{14}C] ornithine hydrochloride and cold L-ornithine (Sigma, final concentration of total L-ornithine, approximately 1.2 m*M*). The reaction was stopped after 90 min at 15°C by injecting 0.5 ml 5% trichloroacetic acid into all tubes. These tubes stood for at least 1 h to permit maximum absorption of CO_2. Control tubes used to determine endogenous $^{14}CO_2$ release were prepared by adding first 0.5 ml TCA followed by 150 μl of ODC supernatant and 30 μl L-ornithine. The filter paper was removed and placed in scintillation vials containing 4.0 ml Beckmann NA scintillation fluid. The vials stood in the dark overnight so that the chemiluminescence would be reduced before the radioactivity was measured in an LKB Excel liquid scintillation counter.

The activity of ODC was measured at seven concentrations ranging from 0.0351 to 1.144 m*M* ornithine at assay temperatures of 0, 5, and 15°C. These temperatures are within the normal range of temperature to which the sea stars are exposed during the year (−2 to 17°C). All of the assays were performed in duplicate. Activities were expressed as nmoles ornithine converted to putrescine per h per g wet weight tissue. The apparent Michaelis constant (K_m) was determined from double reciprocal plots. Although potential problems may arise using crude supernatants to determine enzyme activity, relative differences in enzyme activities should reflect biological differences with respect to changes in temperature.

Results

The activity of ODC increased hyperbolically with an increase in substrate (ornithine) concentration (Fig. 1). The affinity of the enzyme for ornithine decreased with the assay temperature at all substrate concentrations

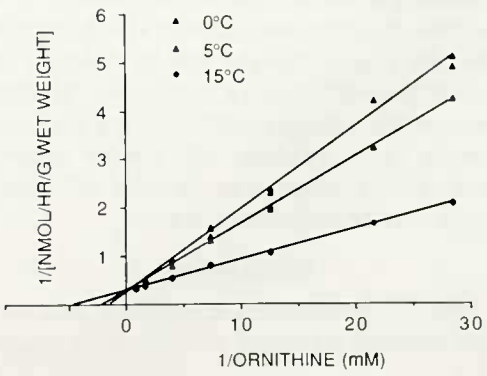

Figure 2. Double-reciprocal plot of the activity of ornithine decarboxylase *versus* substrate concentration. All assays were performed in duplicate.

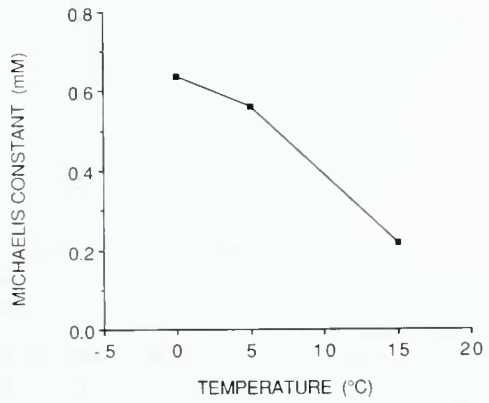

Figure 3. Relation of the apparent Michaelis contant (Km, m*M*), as determined from the double-reciprocal plot of the activity of ornithine decarboxylase, and assay temperature. A decrease in the assay temperature caused an increase in K_m and, therefore, a decrease in the apparent affinity of the enzyme for the substrate.

tested, indicating temperature-dependent enzyme-substrate affinity parameters.

The double reciprocal plots (Fig. 2) of ODC activity show that the assay temperature did not affect the maximal velocity of ornithine decarboxylase, as V_{max} did not change with temperature between 0 and 15°C. An increase in the slope of the line was apparent with a decrease in assay temperature, indicating an increase in the apparent K_m. A plot of apparent K_m *versus* temperature, illustrating negative thermal modulation (increased K_m with a decrease in temperature), is shown in Figure 3.

The Q_{10} values for the ODC activities were calculated between both 0 and 5°C and 5 and 15°C (Table I). Q_{10} values were significantly higher (sign test, $P < 0.05$) at the low temperatures at all substrate concentrations, ranging from 2.21 to 2.59 between 0 and 5°C. Q_{10} values were lowest at the high temperatures, ranging from 1.07 to 2.04 between 5 and 15°C. Q_{10} values of approximately unity indicated that ODC activity was essentially temperature-independent at high substrate concentrations (1.14 m*M* ornithine) between 5 and 15°C. These values show that ODC activity is temperature-dependent at low substrate concentrations and at low temperatures (regardless of substrate concentration), and temperature-independent at high substrate concentrations at higher temperatures only.

Discussion

The effects of temperature on organismal behavior, physiology, and the biochemistry of some cellular processes has been well documented for many ectothermic invertebrates. At the cellular level, the influence of temperature on enzyme activities is varied and dependent on the interactions of substrates and cofactors, translational and transcriptional control of enzyme production, and a host of other factors such as the metabolic pathway, type of tissue, organism, and the physical environment to which the organism is acclimatized or adapted (15).

Metabolic pathways influencing the rate of cell and tissue growth (including hyperplastic and hypertrophic growth) include those that influence the rate of energy production (glycolysis, Krebs cycle) and those that influence the rates of macromolecular biosynthesis (proteins, lipids, nucleic acids). Temperature acclimation and adaptation of many of the enzymes involved in energy production have been reviewed by Hazel and Prosser (16). Fewer studies have focused on temperature adaptation of those enzymatic reactions directly involved in macromolecule synthesis, particularly those involved in regulating macromolecule synthesis and function.

Polyamine biosynthesis, regulated by the activity of ODC, is directly involved in macromolecular synthesis and is considered to be one of the rate-limiting steps in the regulation of protein and nucleic acid metabolism, as well as cell growth (1, 2, 17, 18, 19). In this study we have investigated the effects of temperature on the kinetic characteristics of an invertebrate ODC obtained from the testes of the sea star *Asterias vulgaris*. *A. vulgaris* is seasonally exposed to temperatures ranging from −2 to 17°C (11). Watts *et al.* (11) found that significant decreases in testicular growth, polyamine levels (putrescine, spermidine, and spermine), and ODC activity were coincident with decreasing or low environmental temperatures. It was hypothesized that low field temperatures resulted in a decrease in polyamine biosynthesis by directly influencing the synthesis of ODC, either at the translational or transcriptional level.

In this experiment we have shown that ODC exhibits "negative thermal modulation" (15). Decreases in assay temperature resulted in increases in the apparent Km, thereby lowering the affinity of the enzyme for the substrate. Reduced enzyme affinity for the substrate, as well

TABLE I

Values of Q_{10} as determined from the activity of ornithine decarboxylase measured at various substrate concentrations

[ORNITHINE] (m*M*)	0–5°C	5–15°C
.0351	2.35	2.04
.0463	2.59	1.94
.0799	2.36	1.86
.1359	2.34	1.69
.2479	2.29	1.45
.5839	2.26	1.26
1.144	2.21	1.07

Values of Q_{10} were determined over the range of 0 to 5°C and 5 to 15°C.

as reduced ODC synthesis reported previously by Watts *et al.* (11) suggest that polyamine biosynthesis, which is necessary for cell and tissue growth, may be decreased during exposure to low temperature, thereby inhibiting growth.

The influence of temperature on the rate of ODC activity becomes more apparent when examined in terms of Q_{10}. The combined effects of reduced substrate binding and reduced kinetic energy available for enzyme activation at low temperatures produced higher Q_{10} values at apparent physiological substrate concentrations (*ca.* 0.2 m*M* ornithine; Watts, unpub.). In addition, Q_{10} values are highest when calculated at the reduced assay temperatures, causing temperature-dependent ODC activity and kinetics at low temperatures.

We hypothesize that the exposure of *Asterias vulgaris* to low temperatures decreases growth and development of the testis by negative modulation of polyamine synthesis. In individuals exposed to low temperatures, we suggest that polyamine synthesis may be negatively modulated by 1: a decrease in the amount of enzyme (either translational or transcriptional control), 2: an increase in the amount or binding of a proposed antizyme (1, 2), or 3: a decrease in the affinity of the enzyme for the substrate as indicated by changes in the K_m. Thermal modulation of polyamine metabolism may be a mechanism by which seasonal temperature fluctuations induce the seasonal patterns of growth observed in many ectothermal organisms. Further studies are needed to determine the extent to which temperature influences these processes in these organisms.

Acknowledgments

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